MSD

Medical Science and Discovery
ISSN: 2148-6832

Detection of Viral Respiratory Factors via Multiplex PCR in Newborn & Pediatric Patients and Their Distribution According to Seasons

Murat Yaman[1]*, Salih Hazar[2], Edanur Yeşil[3], Merve Havan[4]

1 Dept of Medical Virology, Mersin City Training and Research Hospital, Mersin, TR
2 Dept of Medical Microbiology, Mersin City Training and Research, Mersin, TR
3 Dept of Pediatric Infectious Diseases, Mersin City Training and Research Hospital, Mersin, TR
4 Dept of Pediatric Intensive Care, Mersin City Training and Research Hospital, Mersin, TR

*** Corresponding Author:** Murat Yaman **E-mail:** drmrtyaman@gmail.com

ABSTRACT

Objective: Respiratory viruses are a global public health problem, and viruses cause up to 80% of respiratory infections. This study aimed to elucidate the viral respiratory tract factors and the frequency of coinfections in the newborn and pediatric age groups determined by the molecular respiratory tract panel (MRTP) kit.

Materials & Method: The results of the respiratory tract panel test with the molecular multiplex method were applied to 1486 newborn and pediatric patients between 01.10.2020 and 30.04.2022 to determine the viral respiratory tract factors were analyzed retrospectively. The Multiplex RT – PCR test confirmed results were recorded from the hospital database under the supervision of a microbiologist, negative and positive controls were evaluated, and test was validated.

Results: Clinical virology laboratory test results were scanned and at least one respiratory tract virus was detected in nasopharyngeal swabs of 499 (33.6%) patients. A total of 634 viruses were detected in 499 NS-positive samples. The most commonly detected viral pathogens were parainfluenza – 3 (36.9%, n=184), respiratory syncytial virus (22.8%, n=114), human rhinovirus (19.2%, n=96), SARS-CoV-2 (12.6%, n=63), and human bocavirus (10.8%, n=54) respectively.

Conclusion: In this research, we tried to elaborate the accuracy of molecular multiplex method and the respiratory tract panel test to determine the respiratory factors in newborn and pediatric age group patients. The logic behind this lies beneath the fact that diagnosing with a kit that can detect both single and multiple factors causing coinfection can be performed simultaneously.

Keywords: viruses, multiplex RT – PCR, nasopharyngeal swab, respiratory tract infection, newborn, childhood

INTRODUCTION

Respiratory viruses are a global public health problem, and viruses cause up to 80% of respiratory infections. This incidence leads to significant morbidity and mortality worldwide (1, 2). Viral respiratory tract infections are common in newborns and children (3, 4). They constitute approximately half of pediatric community-acquired pneumonia cases and a quarter of adult pneumonia cases (5). Acute respiratory tract infections are considered to be one of the most important causes of death in children <5 years of age (4). Winter and spring are peak seasons for flu and other common respiratory infections (5, 6).

The use of rapid antigen tests with extremely high specificity and low sensitivity is reliable when respiratory viral infections are at a high prevalence. Serological tests are primarily used for epidemiological studies and their application in the diagnosis of viral respiratory tract infections is limited (7, 8). Technological advances in molecular diagnostic tests have started a revolution in the field of diagnostic virology. Molecular methods such as polymerase chain reaction (PCR) and real-time polymerase chain reaction (RT – PCR) are more widely performed than other methods for the laboratory diagnosis of viral respiratory tract infections and can be used in conventional methods such as cell culture. Because they show high sensitivity and specificity, obtain results in a short time, and identify viruses that are difficult to culture or slow to reproduce. Today, many multiplex RT – PCR tests have been developed to diagnose an increasing number of respiratory tract viruses, in which more than one virus can be detected simultaneously in a single test (9, 10).

Research Article

Received 10-10-2022

Accepted 21-10-2022

Available Online: 22-10-2022

Published 30-10-2022

http://dx.doi.org/10.36472/msd.v9i10.826

This study aimed to elucidate the viral respiratory tract factors and the frequency of coinfections in the newborn and pediatric age groups determined by the molecular respiratory tract panel (MRTP) kit and to investigate the seasonal distribution with demographic data such as age and gender.

MATERIAL and METHODs

The results of the respiratory tract panel test with the molecular multiplex method, which was applied to 1486 newborn and pediatric patients between 01.10.2020 and 30.04.2022 in order to determine the viral respiratory tract factors, were analyzed retrospectively. The ethics committee approval was granted on 29/04/2022 with protocol number: 4/87. The study complied with the Declaration of Helsinki, and informed consent was obtained from all participants.

Nasopharyngeal and throat nasopharyngeal swab samples, containing a viral nucleic acid extracting and protective liquid have been collected from from pediatric patients with respiratory symptoms. These materials were delivered to the microbiology laboratory with a transfer tube. After the nucleic acid was obtained, the RT – PCR result was gained by processing the protocol specified by the manufacturer. Negative and positive controls were carefully evaluated, and the test was validated.

Statistical Analysis: SPSS 25 (SPSS Inc, Chicago, IL, USA) package program was used for statistical evaluation of the data. Continuous data were given as the median. Categorical data were expressed as numbers and percentages. Visual features (histogram and probability plots) and Kolmogorov-Smirnov test were utilized for the normal distribution of the variables. Student's T test or Mann – Whitney U test was used for comparison. The Mann – Whitney U test was utilized to compare continuous variables. Qualitative variables were compared via Pearson Chi-Square or Fisher exact tests. A P value <0.05 was considered statistically significant.

RESULTS

RT – PCR results of the nasopharyngeal swab (NS) samples taken from 1486 children aged 0 – 18 years who were diagnosed with acute respiratory tract infection between October 2020 and April 2022 were included in this study. The gender distribution was 58.6% (n=871) of the children were male, and 41.4% (n=615) were female. The median age was 2 years (ranging between 0 – 18 years).

The most commonly detected viral pathogens were Parainfluenza – 3 (PIV3) (36.9%, n=184), RSV (22.8%, n=114), HRV (19.2%, n=96), SARS-CoV-2 (12.6%, n=63), and HBoV (10.8%, n=54) respectively. The frequency of respiratory viral pathogens detected by RT – PCR is presented in **Table 1**.

Single viral infections were detected in 442 (29.7%) of 1486 samples, and two or more viral infections were found in 57 samples (3.8%). The most common multiple infections were found to be HRV and PIV3 (12.3%, n=7) (**Table 2**).

Viral agents were detected in 499 of the 1486 patient samples with the diagnosis of respiratory tract infection, of which 292 (33.5%) were male, and 297 (33.7%) were female. No statistically significant difference was found when the gender distribution of single and multiple infections was evaluated. PIV3, RSV, HRV were the most common viral agents in both male and female patients. Distribution of viruses by gender in respiratory tract samples is presented in **Table 3**.

Viral respiratory pathogens were observed at the highest rate in the 0 – 1 year-old age group (37.9%, n=245). The other distribution was as follows: >1 – 5 years old (34.4%, n=185), >5 – 10 years old (26.1%, n=37) and >10 – 18 years old (20.1%, n=32). While RSV (15.3%) was the most common factor in the 0 – 1 age group ($p<0.001$), it was determined that PIV3 was the most frequent viral pathogen in other age groups. RSV (15.3%), PIV3 (14.2%) and HRV (8.3%) were the most common respiratory tract viruses at ≤1 year of age. Single infection was more in the 0 – 1 year age group (33.5%, n=217), while multiple infections were found in the >1 – 5 years age group (4.6%, n=25) ($p<0.001$) (**Table 4**).

During the study period, the respiratory test positivity rate was found to be the highest in spring (February – March – April) (61.4%, n=226) ($p<0.001$). At the same time, it was found that 215 (58.4%) of the patients were infected with a single virus in this season, and 11 (3.0%) were infected with at least two viruses.

When each virus's seasonal and monthly distribution was analyzed, it was observed that PIV3, RSV, HRV, and HBoV were detected in all four seasons. HRV (9.1%) was the most common cause in winter, and adenovirus (AV) in summer. The seasonal distribution of viral agents was elaborated in **Table 5**.

http://dx.doi.org/10.36472/msd.v9i10.826

Table 1. Demographic and laboratory characteristics of the patients

		%	n	p
Viral pathogen positive patient	Total	33.6	499/1486	
	Male	33.5	292/871	0.957
	Female	33.7	207/615	
Median Age (years)			2 (0-18)	
Single infection		29.7	442/1486	
Multipl infection		3.8	57/1486	
	AV, HBoV	1.8	1/57	
	AV, HRV	3.5	2/57	
	AV, HCoV HKU1	1.8	1/57	
	AV, PIV3	5.3	3/57	
	HCoV 229E, HRV	1.8	1/57	
	HCoV HKU1, EV	1.8	1/57	
	HCoV OC43, HCoV NL63	1.8	1/57	
	HCoV OC43, PIV3	1.8	1/57	
	HCoV OC43, RSV	5.3	3/57	
	HCoV OC43, HRV	1.8	1/57	
	HBoV, EV	1.8	1/57	
	HBoV, PIV3	1.8	1/57	
	HBoV, Sars CoV-2	1.8	1/57	
	INF-A, HBoV	1.8	1/57	
	INF-A, RSV	1.8	1/57	
	INF-A, Sars CoV-2	1.8	1/57	
	HpeV, PIV3	3.5	2/57	
	PIV2, PIV3	1.8	1/57	
	RSV, HBoV	5.3	3/57	
	RSV, PIV3	8.8	5/57	
	RSV, HRV	3.5	2/57	
	HRV, EV	1.8	2/57	
	HRV, HBoV	1.8	1/57	
	HRV, PIV3	12.3	7/57	
	HRV, Sars CoV-2	1.8	1/57	
	Sars CoV-2, EV	1.8	1/57	
	Sars CoV-2, PIV3	5.3	3/57	
	AV, HCoV 229E, HCoV OC43	1.8	1/57	
	HCoV OC43, HCoV HKU1, PIV3	1.8	1/57	
	HBoV, PIV1, PIV3	1.8	1/57	
	hMPV, HpeV, HRV	1.8	1/57	

HpeV: human parechovirus; HRV: human rhinovirus; INF-A: influenza A virus; hMPV: human metapneumovirus; HBoV: human bocavirus; HCoV: human coronavirus; PIV: parainfluenza virus AV: adenovirus; RSV: respiratory syncytial virus; EV: Enterovirus. Data are given as number (%).

http://dx.doi.org/10.36472/msd.v9i10.826

Table 2. Distribution of respiratory viral pathogen samples (n=499) detected by RT – PCR

Specific viral pathogen	N	%
Adenovirus	27	5.4
Bocavirus	54	10.8
Corona 229 E	9	1.8
Corona HKU1	8	1.6
Coronavirus NL63	3	0.6
Coronavirus OC43	18	3.6
Enterovirus	7	1.4
Human metapneumovirus A/B	11	2.2
Influenza A	26	5.2
Influenza B	0	0
Influenza A-H1	3	0.6
Parechovirus	6	1.2
Parainfluenza 1	1	0.2
Parainfluenza 2	4	0.8
Parainfluenza 3	184	36.9
Parainfluenza 4	0	0
RSV	114	22.8
Rhinovirus	96	19.2
Sars-CoV-2	63	12.6
Total	634	100

Table 3. Distribution of viruses by gender in respiratory tract samples

	Male (n=871)	Female (n=615)	Total (n=1486)	P value
Single infection	259 (29.7)	183 (29.8)	442 (29.7)	0.993
Multipl infection	33 (3.8)	24 (3.9)	57 (3.8)	
Positive Respiratory Test	292 (33.5)	297 (33.7)	499 (33.6)	0.957
Viral pathogen				
AV	22 (2.5)	5 (0.8)	27 (1.8)	0.015
HBoV	31 (3.6)	23 (3.7)	54 (3.6)	0.855
HCoV 229 E	4 (0.5)	5 (0.8)	9 (0.6)	0.502
HCoV HKU1	4 (0.5)	4 (0.7)	8 (0.5)	0.725
HCoV NL63	1 (0.1)	2 (0.3)	3 (0.2)	0.573
HCoV 0C43	10 (1.1)	8(1.3)	18 (1.2)	0.791
EV	2 (0.2)	5 (0.8)	7 (0.5)	0.106
hMPV	11 (1.3)	0 (0)	11 (0.7)	0.004
INF-A	9 (1.0)	17 (2.8)	26 (1.7)	0.012
INF-B	0 (0)	0(0)	0(0)	
INF-A-H1	2 (0.2)	1 (0.2)	3 (0.2)	0.777
HpeV	3 (0.3)	3 (0.5)	6 (0.4)	0.696
PIV1	0 (0)	1 (0.2)	1 (0.1)	0.414
PIV2	1 (0.1)	3 (0.5)	4 (0.3)	0.313
PIV3	108 (12.4)	76 (12.4)	184 (12.4)	0.981
PIV4	0	0	0	
RSV	68 (7.8)	46 (7.5)	114 (7.7)	0.815
HRV	65 (7.5)	31 (5)	96 (6.5)	0.061
SARS-CoV-2	34 (3.9)	29 (4.7)	63 (4.2)	0.444

HpeV: human parechovirus; HRV: human rhinovirus; INF-A: influenza A virus; hMPV: human metapneumovirus; HBoV: human bocavirus; HCoV: human coronavirus; PIV: parainfluenza virus AV: adenovirus; RSV: respiratory syncytial virus; EV: Enterovirus. Data are given as number (%).

http://dx.doi.org/10.36472/msd.v9i10.826

Tablo 4. Distribution of viruses by age groups in respiratory tract samples

Viral Pathogen	Age Groups 0-1 n = 647	>1-5 n = 538	>5-10 n = 142	>10-18 n = 159	Total n = 1486	P Value
ADV	5 (0.8)	20 (3.7)	1 (0.7)	1 (0.6)	27 (1.8)	0.001
HBoV	12 (1.9)	38 (7.1)	3 (2.1)	1 (0.6)	54 (3.6)	< 0.001
HCoV 229 E	7 (1.1)	2 (0.4)	0 (0.0)	0 (0.0)	9 (0.6)	0.320
HCoV HKU1	3 (0.5)	4 (0.7)	1 (0.7)	0 (0.0)	8 (0.5)	0.690
HCoV NL63	1 (0.2)	1 (0.2)	1 (0.7)	0 (0.0)	3 (0.2)	0.521
HCoV 0C43	8 (1.1)	9 (1.7)	1 (0.7)	0 (0.0)	18 (1.2)	0.430
EV	3 (0.5)	2 (0.4)	2 (1.4)	0 (0.0)	7 (0.5)	0.332
hMPV	8 (1.2)	2 (0.4)	0 (0.0)	1 (0.6)	11 (0.7)	0.303
INF-A	9 (1.4)	12 (2.2)	2 (1.4)	3 (1.9)	26 (1.7)	0.723
INF-B	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
INF-A-H1	0 (0.0)	2 (0.4)	1 (0.7)	0 (0.0)	3 (0.2)	0.144
HpeV	2 (0.3)	3 (0.6)	0 (0.0)	1 (0.6)	6 (0.4)	0.835
PIV1	0 (0.0)	1(0.2)	0 (0.0)	0 (0.0)	1 (0.1)	0.565
PIV2	2 (0.3)	2 (0.4)	0 (0.0)	0 (0.0)	4 (0.3)	0.622
PIV3	92 (14.2)	64 (11.9)	12 (8.5)	16 (10.1)	184 (12.4)	0.176
PIV4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
RSV	99 (15.3)	14 (2.6)	1 (0.7)	0	114 (7.7)	< 0.001
HRV	54 (8.3)	35 (6.5)	5 (3.5)	2 (1.3)	96 (6.5)	0.005
SARS-CoV-2	33 (5.1)	12 (2.2)	8 (5.6)	10 (6.3)	63 (4.2)	0.031
Pathogen Number						
Single infection	217 (33.5)	160 (29.7)	36 (25.4)	29 (18.2)	442 (29.7)	< 0.001
Multipl infection	28 (4.3)	25 (4.6)	1 (0.7)	3 (1.9)	57 (3.8)	
Total	245 (37.9)	185 (34.4)	37 (26.1)	32 (20.1)	499 (33.6)	< 0.001

Table 5. Respiratory viruses and seasonal distribution

	Spring n= 368	Summer n= 148	Autumn n= 333	Winter n= 637	P Value
Single infection	215 (58.4)	60 (40.5)	73 (21.9)	94 (14.8)	<0.001
Multipl infection	11 (3.0)	17 (11.5)	20 (6.0)	9 (1.4)	
Viral pathogen					
ADV	0	26 (17.6)	1 (0.3)	0	<0.001
HBoV	29 (7.9)	20 (13.5)	2 (0.6)	3 (0.5)	<0.001
HCoV 229 E	0 (0)	9 (6.1)	0 (0)	0 (0)	<0.001
HCoV HKU1	3 (0.8)	5 (3.4)	0 (0)	0 (0)	<0.001
HCoV NL63	0 (0)	3 (2.0)	0 (0)	0 (0)	0.001
HCoV 0C43	0 (0)	18 (12.2)	0 (0)	0 (0)	<0.001
EV	1 (0.3)	3 (2.0)	3 (0.9)	0 (0)	0.003
hMPV	11(3.0)	0 (0)	0 (0)	0 (0)	<0.001
INF-A	26 (7.1)	0 (0)	0 (0)	0 (0)	<0.001
INF-B	0 (0)	0 (0)	0 (0)	0 (0)	
INF-A-H1	3 (0.8)	0 (0)	0 (0)	0 (0)	0.069
HpeV	6 (1.6)	0 (0)	0 (0)	0 (0)	0.001
PIV1	1 (0.3)	0 (0)	0 (0)	0 (0)	0.571
PIV2	4(1.1)	0 (0)	0 (0)	0 (0)	0.016
PIV3	151(41)	6(4.1)	11 (3.3)	16 (2.5)	<0.001
PIV4	0 (0)	0 (0)	0 (0)	0 (0)	
RSV	62(16.8)	3 (2.0)	3(0.9)	46 (7.2)	<0.001
HRV	1 (0.3)	4(2.7)	33 (9.9)	58 (9.1)	<0.001
SARS-CoV-2	2 (0.5)	0 (0)	61(18.3)	0 (0)	<0.001

DISCUSSION

Respiratory viruses play an important role in LRTI during infancy and childhood. Routine laboratory tests and radiological examinations cannot differentiate between viral and bacterial, and empirical antibiotic therapy is mostly given (10). Gonzalez – Carrasco et al. (11)emphasized that viral respiratory tract infections should also be considered in newborns with a runny nose, apnea, and high oxygen requirement, and suspected sepsis. In another study, it has been shown that viral tests can make a significant contribution to elucidating the etiology in infants under 3 months of age presenting with unfocused fever (12). Kidszun et al. (13) reported that in some of the newborns who showed clinical worsening and were thought to have late-onset sepsis in the ICU, no growth was found in the blood culture, and respiratory viruses were detected in the nasopharyngeal aspirate.

In previous literature, it has been reported that RSV was the most common cause of LRTI in infants (14 – 17). RSV was the most common factor with 80% of patients hospitalized in the neonatal ICU due to LRTI. This rate was found to be 80% in the study of Cho et al. (16) and 95% in the study of Bukhari et al. (17).

Designating a single factor can be considered an advantage in terms of precautions and preventive treatments. As it is known, RSV is transmitted mostly by direct contact and contaminated items, and to a lesser extent by droplets. Homaira et al. (18) isolated the ribonucleic acid molecule of RSV from visitors' clothes and frequently used surfaces in the ICU, and it was shown that this was important in contamination.

Due to its high sensitivity and specificity for the diagnosis of respiratory tract viruses, the multiplex RT – PCR test is the method of choice today (19). Lin et al. (2020) stated that RT – PCR had higher detection rates compared with traditional antigen tests and viral cultures (75.3% versus 48.3%). They have detected that RSV, RV, and PIV3 were the leading pathogens detected in pediatric RTI patients (19). In this study, single viral infections were detected in 29.7% of 1486 samples, two or more (multipl) viral infections were found in 3.8% of samples. The most common multiple infections were found to be HRV and PIV3 with a ratio of 12.3%.

Jansen et al. (20) aimed to investigate respiratory tract viruses with multiplex RT – PCR method, using nasopharyngeal aspirate samples from 133 pediatric patients admitted for acute respiratory infection during the winter of 2007 – 2008 in the Netherlands. With the Multiplex RT – PCR test, positive results for one or more viruses were obtained in 68% of samples. Single infection was detected in 50% of the samples, and dual infection in 17%. Rhinovirus was the most common pathogen detected with a rate of 27% followed by RSV 16.5%, adenovirus 10.5%, influenza A 6.7%, hMPV 6%, HBoV 3%, HCoV 2.2%, influenza B 2.2%.

In this study, we found at least one respiratory tract virus in 33.6% patients diagnosed with respiratory tract infection by RT – PCR method. A total of 634 viruses were detected in 499 NS-positive samples. The most commonly detected viral pathogens were parainfluenza–3 (PIV3) (36.9%), respiratory syncytial virus (RSV) (22.8%), human rhinovirus (HRV) (19.2%), SARS-CoV-2 (12.6%), and human bocavirus (HBoV) (10.8%4) respectively.

Özcan et al. (21) investigated the presence of viral respiratory pathogens using multiplex RT – PCR in 104 children aged 3–17 years who had asthma attacks. Respiratory viruses were detected in 53.8% of the nasopharyngeal and nasal swab samples. The most common viral agent was rhinovirus (35.6%). Although viral URTIs are the most common cause of asthma attacks, it has been stated that the severity of exacerbation was independent of the presence of respiratory virus.

Beka et al. (22) tested samples of 109 children with acute respiratory tract infections for RSV, rhinovirus, influenza virus, hMPV, adenovirus, PIV and HCoV by multiplex PCR test in their study in Istanbul. Respiratory viruses were detected in 39.4% of the cases (rhinovirus 14.7%, RSV B 7.3%, influenza A 6.4%, hMPV 3.6%, adenovirus 3.6%, HCoV 0.9%, PIV 3 0.9%, PIV 4% 0.9 and RSV A were found to be 0.9%). For the diagnosis of RSV infections, the sensitivity of PCR and DFA testing was 100% and 100%, and the specificity was 97% and 100%, respectively.

The main limitation of this research may be attributed to its retrospective nature. On the contrary, the high number of sample size, and segmentation of patients according to age and seasonal effects can be counted as the study's strengths.

CONCLUSION

In this research, we tried to elaborate the accuracy of molecular multiplex method and the respiratory tract panel test to determine the respiratory factors in newborn and pediatric age group patients. The logic behind this lies beneath the fact that diagnosing with a kit that can detect both single and multiple factors causing coinfection can be performed simultaneously.

Acknowledgments: None.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions: MY, SH, EY, MH; Study, Design, Data Collection and/or Processing, Analysis and/ or Interpretation **MY**; Writing, and Revisions

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The ethics committee approval has been granted at 29/04/2022 with protocol number: 4/87. The study complied with the Declaration of Helsinki and informed consent has been obtained from all participants.

Abbreviations

AV : Adenovirus
DFA : Direct Fluorescent Antibody
EIA : Enzyme Immunoassay
HBoV : Human Bocavirus
HCoV : Human Coronavirus
HEV : Human Enterovirus
HPeV : Human Parechovirus
HRV : Human Rhino Virus
ICU : Intensive Care Unit
LRTI : Lower Respiratory Tract Infection
MPV : Metapneumovirus
MRTP : Molecular Respiratory Tract Panel
NS : Nasopharyngeal Swab
PIV : Parainfluenza virus
RSV : Respiratory Syncytial Virus
RT – PCR : Real Time- Polymerase Chain Reaction
SPSS : Statistics Package for Social Sciences

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